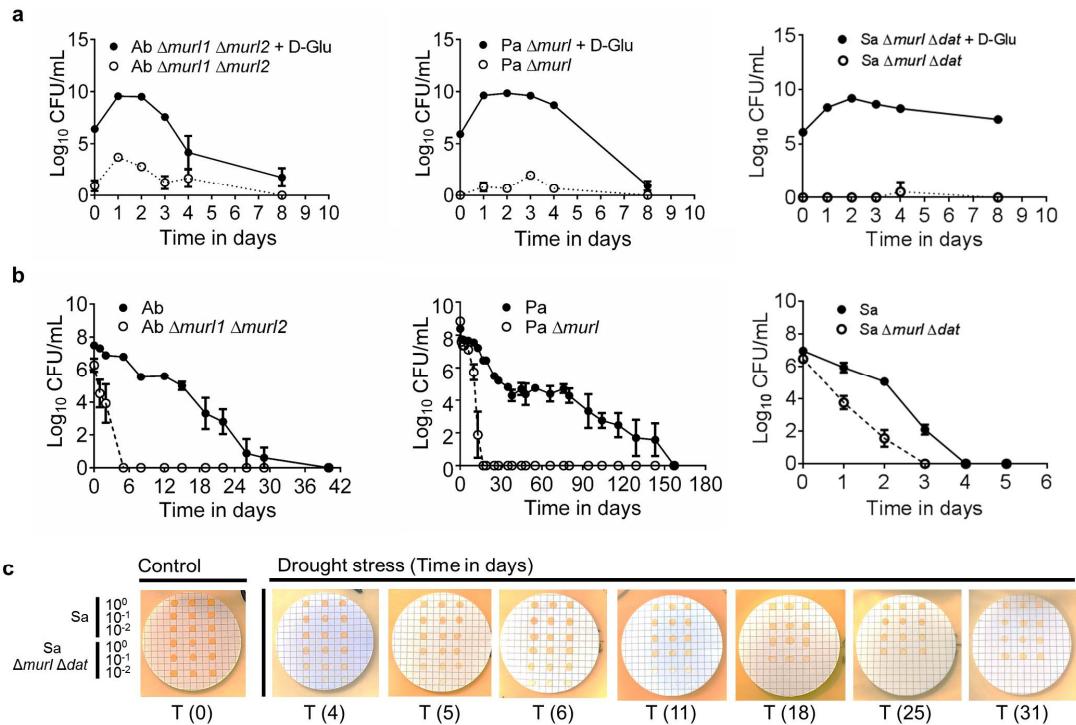
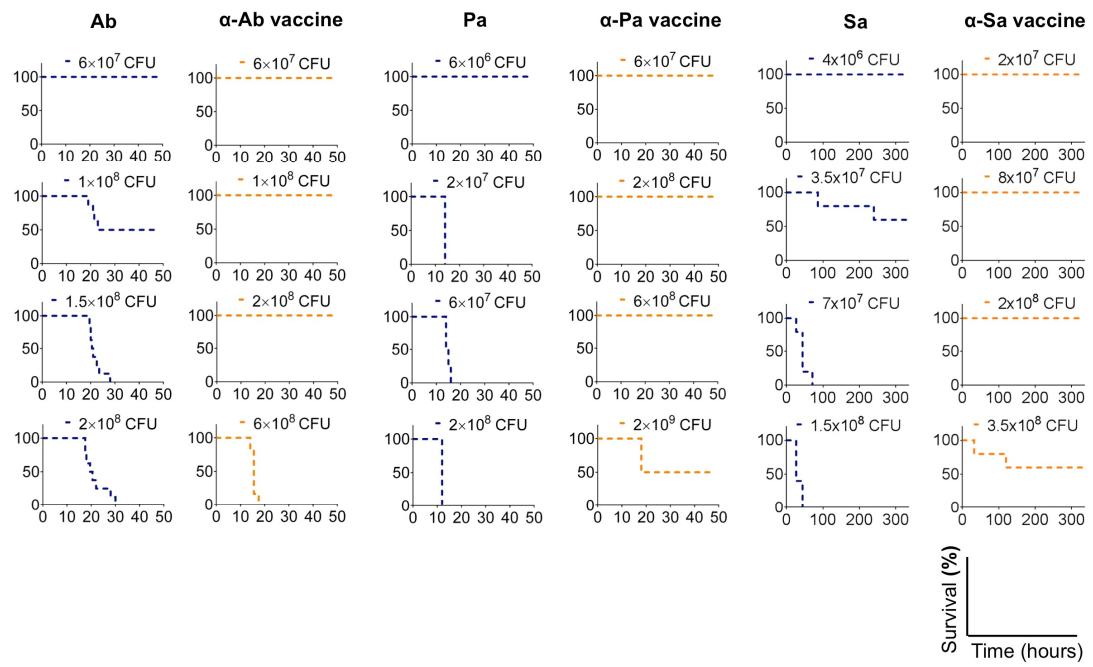


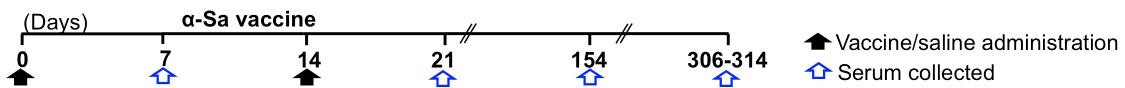
Supplementary Figure 1 *A. baumannii* MurI⁻ and *S. aureus* MurI⁻Dat⁻ altered pattern of cell division. SEM of *A. baumannii* ATCC 17978 (Ab), Ab $\Delta murI1 \Delta murI2$, *S. aureus* 132 (Sa) and Sa $\Delta murI \Delta dat$ in the presence of 0.1 mM D-Glu showing differences in bacterial cell morphology and an altered pattern of cell division.



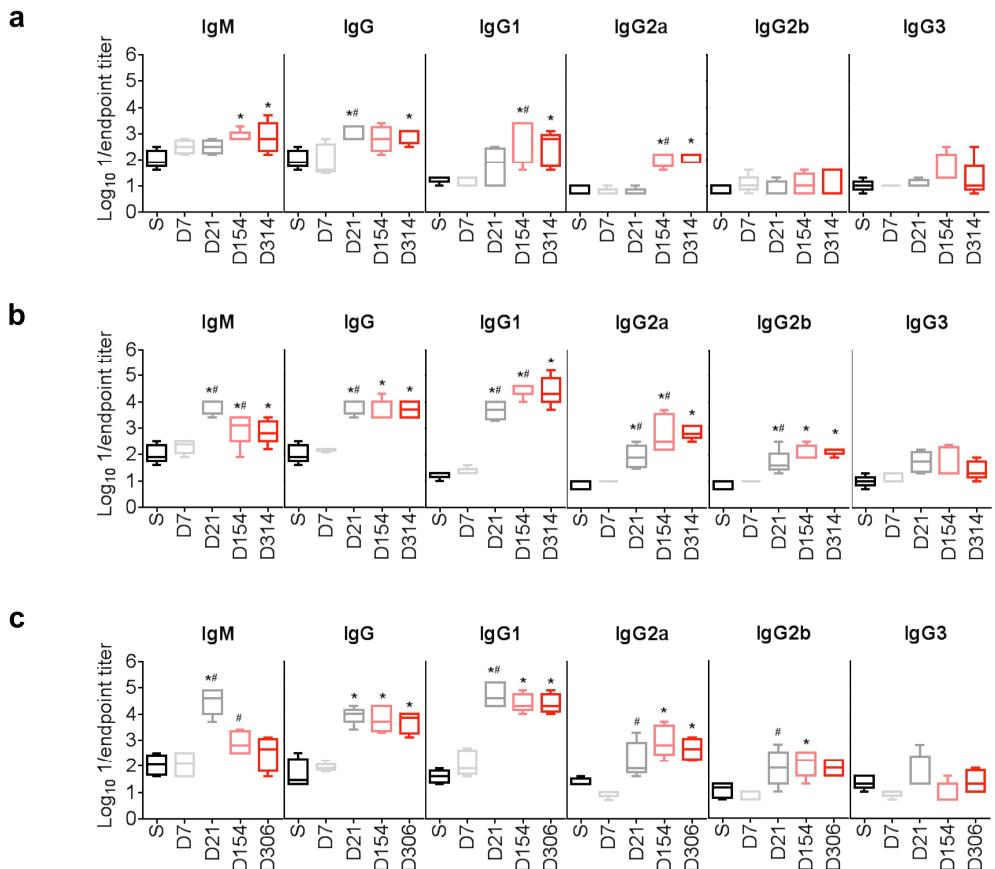
Supplementary Figure 2 D-Glu auxotrophic strains cannot revert to the wild-type phenotype and show lower persistence. **(a)** Viable counts of *A. baumannii* ATCC 17978 (Ab) $\Delta murlI \Delta murl2$, *P. aeruginosa* PAO1 (Pa) $\Delta murlI$, and *S. aureus* 132 (Sa) $\Delta murlI \Delta dat$ obtained on agar plates (\circ) and agar supplemented with 10 mM D-Glu (\bullet) after cultivation on media supplemented with 10-20 mM D-Glu during 8 days. **(b)** Viable counts of Ab, Ab $\Delta murlI \Delta murl2$, Pa, Pa $\Delta murlI$, Sa and Sa $\Delta murlI \Delta dat$ recovered from water during 40, 157 and 5 days (mean \pm s.e.m.). **(c)** Viability of spotted cultures of Sa and Sa $\Delta murlI \Delta dat$ obtained on agar plates supplemented with 10 mM D-Glu at day 0 (control) and after being kept under desiccation conditions during 31 days. **(a, b)** All cultures were made in triplicate (mean \pm s.e.m).



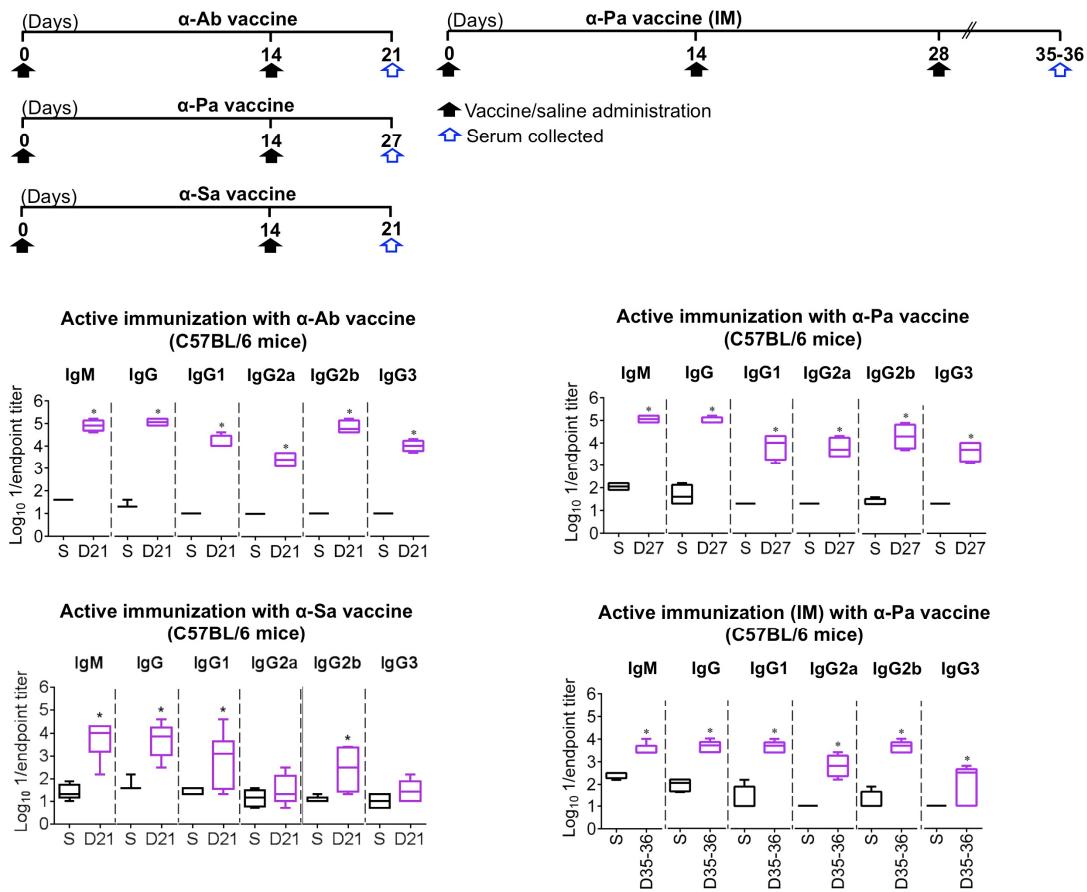
Supplementary Figure 3 D-Glu auxotrophic strains are attenuated compared to parental strains. Survival of BALB/c mice inoculated IP with *A. baumannii* ATCC 17978 (Ab) ($n = 8$), ATCC 17978 $\Delta murI$ $\Delta murI2$ (α -Ab vaccine) ($n = 6$), *P. aeruginosa* PAO1 (Pa) ($n = 4$), PAO1 $\Delta murI$ (α -Pa vaccine) ($n = 4$), *S. aureus* 132 (Sa) ($n = 5$) and 132 $\Delta murI \Delta dat$ (α -Sa vaccine) ($n = 4-5$) with different bacterial doses (CFU as indicated).



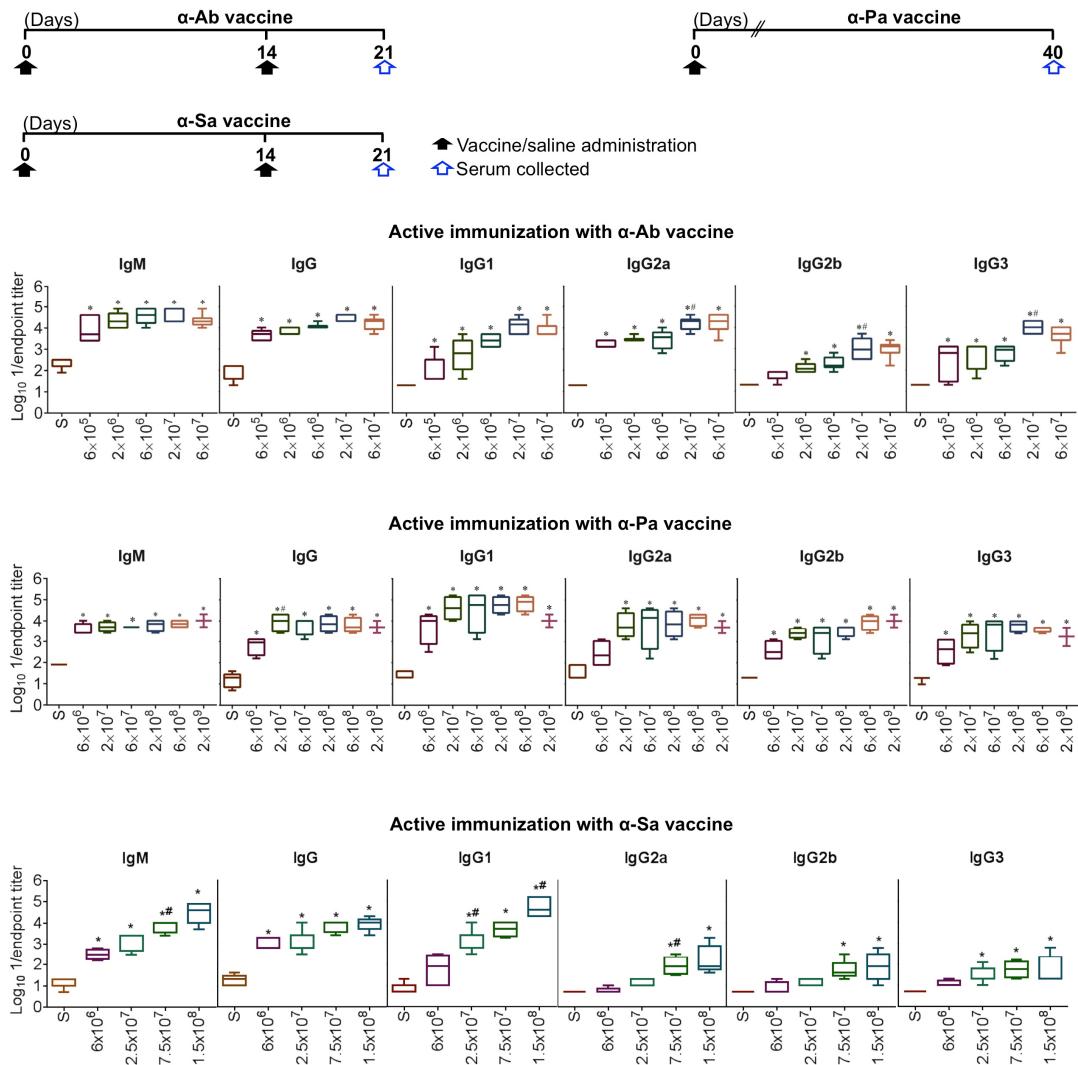
Active immunization with α-Sa vaccine



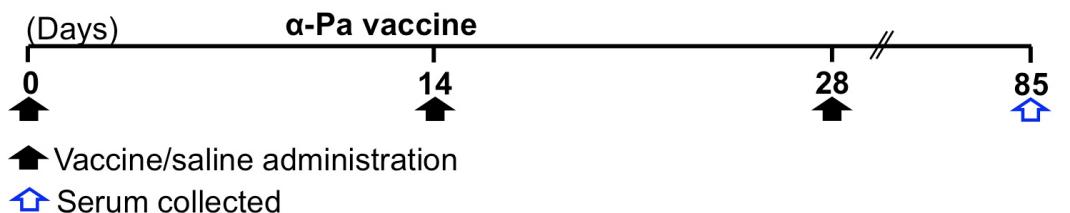
Supplementary Figure 4 Vaccination with *S. aureus* 132 Δ*murI* Δ*dat* (α-Sa vaccine) induces a long-term specific antibody response. Antibody titers elicited in mice ($n = 4-5$) on day 7 (after one immunization) and on days 21, 154 and 306-314 (after two immunizations) by administration of (a) 6×10^6 , (b) 7.5×10^7 and (c) 1.5×10^8 CFU of α-Sa vaccine, or saline. S, saline; D, day. * $P < 0.05$ (Student's t test), compared with saline group. # $P < 0.05$ (one-way ANOVA followed by Bonferroni's post hoc test), compared with antibody production of an immediate lower dose.



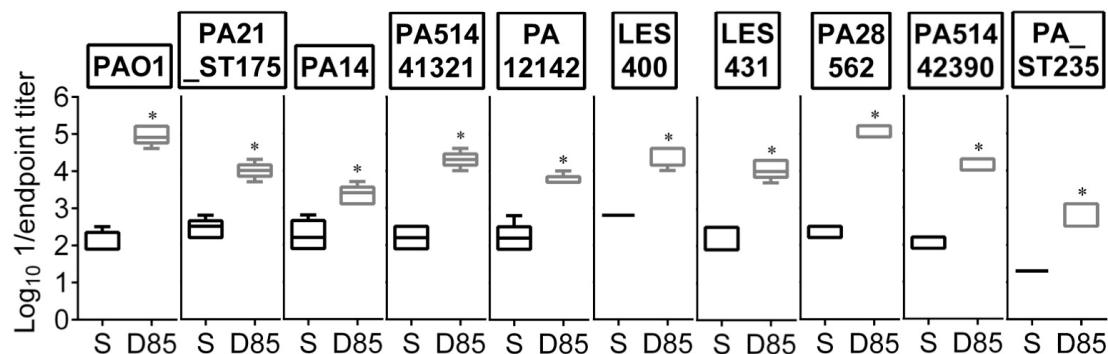
Supplementary Figure 5 Vaccination with D-Glu auxotrophic strains elicits specific antibodies in C57BL/6 mice. Antibody titers in vaccinated and control mice against: *A. baumannii* ATCC 17978 ($n = 3-4$) after two injections with ATCC 17978 $\Delta murII \Delta murI2$ (α -Ab vaccine) (6×10^7 CFU); *P. aeruginosa* PAO1 ($n = 4$) after two injections with PAO1 $\Delta murI$ (α -Pa vaccine) (2×10^7 CFU); *P. aeruginosa* PAO1 ($n = 4-5$) after three intramuscular (IM) injections with α -Pa vaccine (2×10^7 CFU); and *S. aureus* 132 Δspa ($n = 8$) after two injections with 132 $\Delta murI \Delta dat$ (α -Sa vaccine) (3×10^7 CFU), or saline administration, respectively. S, saline; D, day. * $P < 0.05$ (Student's t test), compared with saline group.



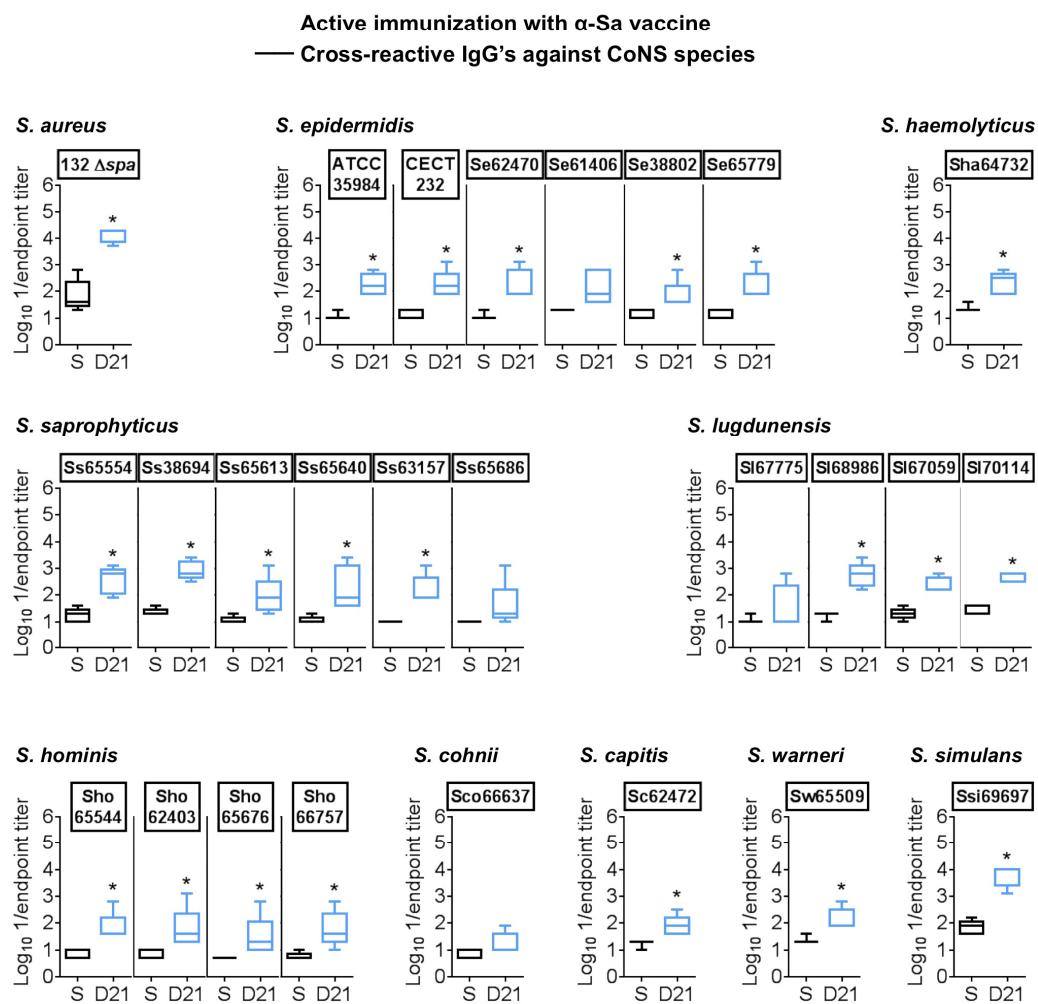
Supplementary Figure 6 Antibody-mediated immune response activated by D-Glu auxotrophic strains is dose-dependent. Antibody titers elicited in mice by administration of different doses (CFU) of *A. baumannii* ATCC 17978 $\Delta murI$ $\Delta murI2$ (α -Ab vaccine) ($n = 5-10$), *P. aeruginosa* PAO1 $\Delta murI$ (α -Pa vaccine) ($n = 2-7$) and *S. aureus* 132 $\Delta murI$ Δdat (α -Sa vaccine) ($n = 4-7$) on days 21 (after two immunizations), 40 (one immunization) and 21 (two immunizations), respectively. * $P < 0.05$ (Student's t test), compared with saline group (S). # $P < 0.05$ (one-way ANOVA followed by Bonferroni's post hoc test), compared with antibody production of an immediate lower dose.



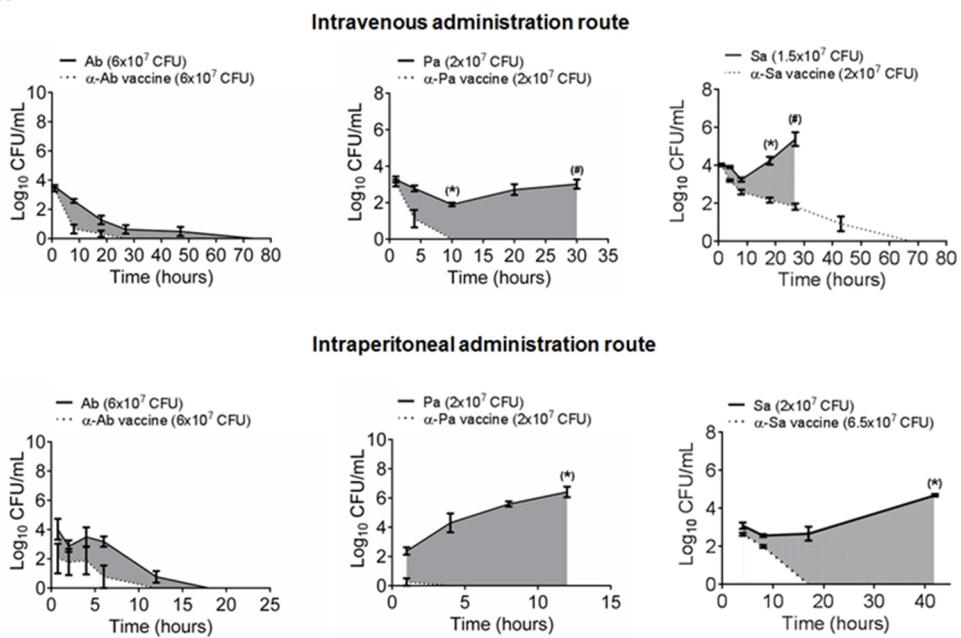
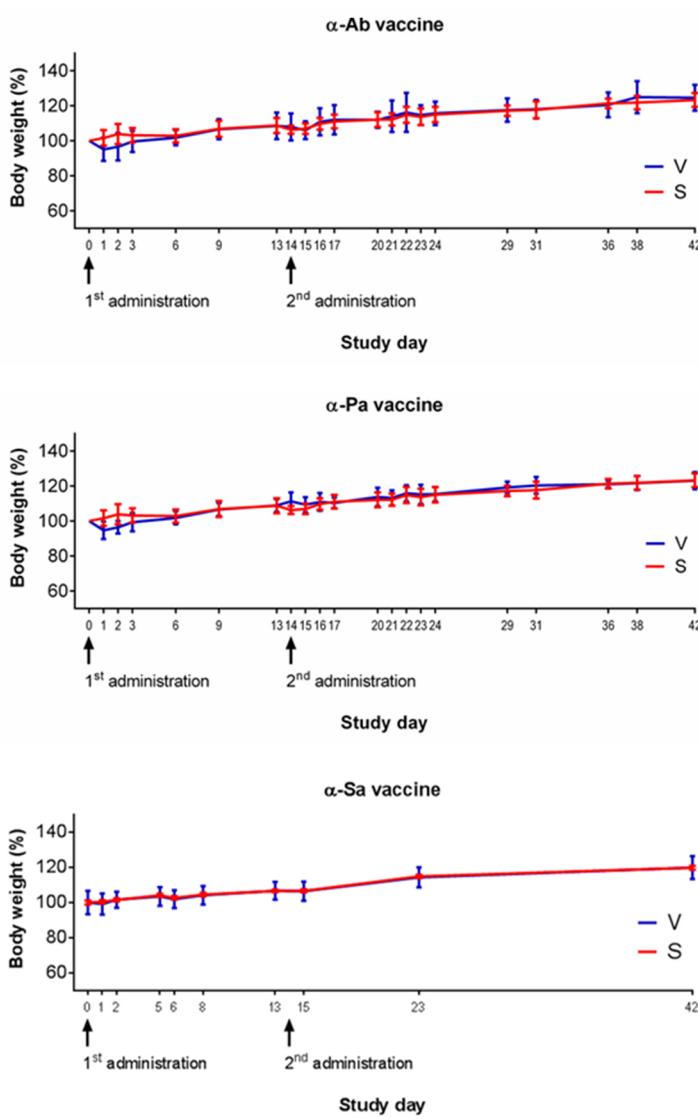
Active immunization with α-Pa vaccine — Cross-reactive IgG's



Supplementary Figure 7 Vaccination with *P. aeruginosa* PAO1 Δ murI (α -Pa vaccine) elicits cross-reactive antibodies. IgG titers against different *P. aeruginosa* strains in vaccinated and control mice ($n = 5$) after three injections with α -Pa vaccine (2×10^7 CFU) or saline, respectively. S, saline; D, day. * $P < 0.05$ (Student's t test), compared with saline group.



Supplementary Figure 8 Vaccination with *S. aureus* 132 Δ murI Δ dat (α -Sa vaccine) elicits cross-reactive antibodies against coagulase-negative *Staphylococci* (CoNS). IgG titers against different CoNS strains in vaccinated and control mice ($n = 5$) after two injections with α -Sa vaccine (3×10^7 CFU) or saline, respectively. S, saline; D, day. * $P < 0.05$ (Student's t test), compared with saline group.

a**b**

Supplementary Figure 9 *In vivo* safety profiles of the D-Glu auxotrophic vaccine candidates. **(a)** Kinetics of blood clearance showing the complete elimination of bacterial vaccines after intravenous and intraperitoneal administration in mice. *A. baumannii* ATCC 17978 (Ab) ($n = 3\text{-}6$), Ab $\Delta m u r I I \Delta m u r I 2$ (α -Ab vaccine) ($n = 3\text{-}6$), *P. aeruginosa* PAO1 (Pa) ($n = 4\text{-}5$), Pa $\Delta m u r I$ (α -Pa vaccine) ($n = 4\text{-}5$), *S. aureus* 132 (Sa) ($n = 4\text{-}6$) and Sa $\Delta m u r I \Delta d a t$ (α -Sa vaccine) ($n = 4\text{-}5$) colonies (mean \pm s.e.m.) recovered over time from the blood of BALB/c mice after the intravenous and intraperitoneal injections (CFU as indicated). (*) Mice succumbed to the systemic infection. (#) Mice were sacrificed by welfare reasons. **(b)** Percent of mice weight change after the intraperitoneal injection of α -Ab (6×10^7 CFU) ($n = 9$), α -Pa (2×10^7 CFU) ($n = 9$) and α -Sa (3×10^7 CFU) ($n = 17\text{-}18$) vaccines compared to saline vehicle (mean \pm s.e.m.).

Supplementary Table 1. Strains and plasmids used in the present work.

Strain or plasmid	Relevant features	Source or reference
<i>A. baumannii</i> strains		
ATCC 17978	Reference strain	ATCC
ATCC 17978 <i>ΔmurII</i>	ATCC 17978 derivative, <i>ΔA1S_0380</i>	This study
ATCC 17978 <i>ΔmurI2</i>	ATCC 17978 derivative, <i>ΔA1S_3398</i>	This study
ATCC 17978 <i>ΔmurII ΔmurI2</i>	ATCC 17978 derivative, <i>ΔA1S_0380 ΔA1S_3398</i>	This study
ATCC 19606	Reference strain	Laboratory collection
AbH12O-A2	Multidrug-resistant clinical isolate from an outbreak, Spain, 2006-2008	(1)
Ab307-0294	Encapsulated clinical isolate from blood, Buffalo, NY, 1994	(2)
<i>P. aeruginosa</i> strains		
PAO1	Reference strain	CECT
PAO1 <i>ΔmurI</i>	PAO1 derivative, <i>ΔPA4662</i>	This study
PA14	Hypervirulent strain from burn infection	(3)
PA21_ST175	Multidrug-resistant high-risk clone	(4)
PA12142	Liverpool epidemic strain isolate from cystic fibrosis patient	(5)
PA51441321	A Coruña Hospital isolate from bronchiectasis patient; Mem ^R , Fep ^R	Laboratory collection
PA51442390	A Coruña Hospital isolate from cystic fibrosis patient; mucoid phenotype; Mem ^R	Laboratory collection
LES400	Liverpool epidemic strain from a cystic fibrosis patient with chronic infection	(6)
LES431	Liverpool epidemic strain from a non-cystic fibrosis parent with pneumonia	(6)
PA28562	A Coruña Hospital isolate from bronchiectasis patient; mucoid phenotype;	Laboratory collection
PA_ST235	Clinical strain from peritoneal fluid, epidemic clone, XDR, exoS ⁻ /exoU ⁺ genotype	(7)

Supplementary Table 1 (cont. 1). Strains and plasmids used in the present work.

Strain or plasmid	Relevant features	Source or reference
<i>E. coli</i> strains		
S17-1	<i>recA</i> ⁻ , <i>thi</i> ⁻ , <i>pro</i> ⁻ , <i>hsdR</i> ⁻ (RP4-2Tc::Mu Km::Tn7)	(8)
TG1	<i>supE thi-1</i> Δ(<i>lac-proAB</i>) Δ(<i>mcrB-hsdSM</i>)5, (<i>rK</i> ⁻ <i>mK</i> ⁻)[<i>F'</i> <i>traD36 proAB lacI^aZΔM15</i>]	(9)
DC10β	Δ <i>dcm</i> in the DH10B background [F- <i>mcrA</i> Δ(<i>mrr-hsdRMS-mcrBC</i>) Φ80 <i>dlacZΔM15 lacX74 endA1</i> <i>recA1 deoR</i> Δ(<i>ara, leu</i>)7697 <i>araD139 galU galK nupG rpsL λ-</i>]	(10)
<i>S. aureus</i> strains		
132	MRSA clinical isolate	(11)
132 Δ<i>murI</i>	132 derivative, Δ <i>murI</i>	This study
132 Δ<i>dat</i>	132 derivative, Δ <i>dat</i>	This study
132 Δ<i>murI Δdat</i>	132 derivative, Δ <i>murI Δdat</i>	This study
132 Δ<i>spa</i>	132 derivative, Δ <i>spa</i> protein A-deficient	(11)
RN4220	restriction-deficient NCTC 8325 derivative, <i>rsbU</i> ⁻ , <i>agr</i>	(12)
FPR3757 (USA300LAC)	Community-acquired MRSA strain from wrist abscess; USA300 epidemic clone	(13)
MW2	Community-acquired MRSA strain from 16-month-old girl (septicaemia and septic arthritis); USA400 epidemic clone	(14)
NEWMAN	MSSA strain from human infection	(15)
Sa07997	Clinical strain PVL(+) isolated from bloodstream infection with initial pulmonary focus	Laboratory collection
RF122	ST151 and CC151 strain from bulk milk (Ireland)	(16)
ED133 (formerly 1174)	ST133 and CC133 strain from ovine mastitis (France)	(17)
ED98	ST5 and CC5 strain from broiler chicken (skeletal infection, United Kingdom)	(18)
<i>S. epidermidis</i> strains		
ATCC 35984	Reference strain (Catheter sepsis)	ATCC
CECT 232	Reference strain (Nasal swab)	CECT
Se62470	A Coruña Hospital isolate from peritoneal fluid	Laboratory collection

Supplementary Table 1 (cont. 2). Strains and plasmids used in the present work.

Strain or plasmid	Relevant features	Source or reference
Se61406	A Coruña Hospital isolate from surgical wound	Laboratory collection
Se38802	A Coruña Hospital isolate from urine; Oxa ^R , Amc ^R	Laboratory collection
Se65779	A Coruña Hospital isolate from urine Oxa ^R , Amc ^R , Gen ^R , LvX ^R , Fos ^R	Laboratory collection
<i>S. haemolyticus</i> strains		
Sha64732	A Coruña Hospital isolate from ascetic fluid; Amp ^R , Oxa ^R , Eri ^R , Cip ^R , LvX ^R , Sxt ^R	Laboratory collection
<i>S. saprophyticus</i> strains		
Ss65554	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
Ss38694	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
Ss65613	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
Ss65640	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
Ss63157	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
Ss65686	A Coruña Hospital isolate from urine; Fos ^R	Laboratory collection
<i>S. lugdunensis</i> stains		
SI67775	A Coruña Hospital isolate from peritoneal fluid	Laboratory collection
SI68986	A Coruña Hospital isolate form biopsy; Amp ^R , Fos ^R	Laboratory collection
SI67059	A Coruña Hospital isolate from wound exudate; Amp ^R , Fos ^R	Laboratory collection
SI70114	A Coruña Hospital isolate from pus/abscess	Laboratory collection
<i>S. hominis</i> strains		
Sho65544	A Coruña Hospital isolate from urine	Laboratory collection
Sho62403	A Coruña Hospital isolate from urine; Gen ^R , Fos ^R	Laboratory collection
Sho65676	A Coruña Hospital isolate from urine; Amp ^R , Oxa ^R , Amc ^R , LvX ^R , Sxt ^R	Laboratory collection

Supplementary Table 1 (cont. 3). Strains and plasmids used in the present work.

Strain or plasmid	Relevant features	Source or reference
Sho66757	A Coruña Hospital isolate from breast abscess	Laboratory Collection
<i>S. cohnii</i> strains		
Sco66637	A Coruña Hospital isolate from wound exudate; Ery ^R	Laboratory collection
<i>S. capitis</i> strains		
Sca62472	A Coruña Hospital isolate from peritoneal fluid	Laboratory collection
<i>S. warneri</i> strains		
Sw65509	A Coruña Hospital isolate from sputum	Laboratory collection
<i>S. simulans</i> strains		
Ssi69697	A Coruña Hospital isolate from surgical wound exudate	Laboratory collection
Plasmids		
pMo130	Km ^R ; <i>oriT</i> ⁺ <i>sacB</i> ⁺ <i>xyle</i> ⁺ , gene replacement vector for allelic exchange in Burkholderia; ColE1 <i>ori</i> ,	(19)
pEX18Gm	Gm ^R ; <i>oriT</i> ⁺ <i>sacB</i> ⁺ , gene replacement vector with MCS from pUC18	(20)
pMAD	Amp ^R ; Ery ^R , <i>bgaB</i> ⁺ , <i>E. coli/S. aureus</i> shuttle vector that is temperature –sensitive in <i>S. aureus</i>	(21)

ATCC, American Type Culture Collection; CECT, Spanish Type Culture Collection; PVL, Panton-Valentine Leukocidin; Mem, Meropenem; Fep, Cefepime; Amc, Amoxicillin-clavulanic; Amp, Ampicillin; Cip, Ciprofloxacin; Ery, Erythromycin; Fos, Fosfomycin; Gen, Gentamicin; Lvx, Levofloxacin; Oxa, oxacillin; Sxt, Trimethoprim-Sulphamethoxazole. R, resistant.

Supplementary Table 2. Oligonucleotides and probes designed for the present work.

Analysis, gene or primer	Orientation	Primer sequence (5'-3')	UPL probe
<i>A. baumannii</i> qRT-PCR			
<i>gyrB</i> (A1S_0004)	Forward	tctctagtcaggaagtgggtacatt	
	Reverse	ggttatattcttcacggcaat	76
<i>murI1</i> (A1S_0380)	Forward	ggcactaaaacacctccgtat	
	Reverse	catcttaatgagttgtccacga	145
<i>murI2</i> (A1S_3398)	Forward	gcaatgacttgagcaagca	
	Reverse	aacttttaagttttgccttc	87
<i>P. aeruginosa</i> qRT-PCR			
<i>proC</i> (PA0393)	Forward	cttcgaaggcactgggtggag	
	Reverse	ttattggccaagctgttcg	20
<i>murI</i> (PA4662)	Forward	gagcggatcggtgatttc	
	Reverse	attgcaggccagttaccagag	50
<i>S. aureus</i> qRT-PCR			
<i>gyrB</i>	Forward	cgggtggcgatacaagaat	
	Reverse	gcgtttacaactgtatgaacca	131
<i>murI</i>	Forward	cagcaactgtgttagctttagaat	
	Reverse	gcacctggatcaattacgc	118
<i>dat</i>	Forward	tggtgttagctgaaaggaatcatagc	
	Reverse	accatcgatatctcaacgga	- (*)
Unmarked deletion of <i>A. baumannii murI1</i> and <i>murI2</i> genes			
UP_murI1(NotI)	Forward	ccccggggccgcgggtctgcacccatcgatga	-
UP_murI1(BamHI)	Reverse	ccccggatccgggacccatccaataacctgaatc	-
DOWN_murI1(BamHI)	Forward	ccccggatccggggctctgtttaggcattc	-
DOWN_murI1(SphI)	Reverse	cccccatcgccgcacccatctgttgcatt	-
UP_murI2(NotI)II	Forward	ccccggccgcgggtggtcaggccttgc	-
UP_murI2(BamHI)II	Reverse	ccccggatccgggtacagccgtatgggtt	-
DOWN_murI2(BamHI)	Forward	ccccggatccgggacgcgttacctgttagaa	-
DOWN_murI2(SphI)	Reverse	cccccatcgccgcgcgtacaactaattgg	-
EXTfw_murI1	Forward	gcaatttaggcacttgagg	-
EXTrv_murI1	Reverse	atacgctcagggtgcac	-
INTfw_murI1	Forward	agcctatgtccgtatgg	-
INTrv_murI1	Reverse	tcaaccagtgtgaattgg	-
EXTfw_murI2	Forward	ccgattggaatgattgac	-
EXTrv_murI2	Reverse	agagcattctggtcgaag	-
INTfw_murI2	Forward	tagcaatagaaccagcgg	-
INTrv_murI2	Reverse	ttgtgccgttacagcttc	-
Unmarked deletion of <i>P. aeruginosa murI</i> gene			
UP_murI(HindIII)II	Forward	cccaagcttggggcaatccggcgatatac	-
UP_murI(NotI)	Reverse	ccccggggccgcggggcgttgcggcagacgg	-
DOWN_murI(NotI)	Forward	ccccggccgcgggtcggtccgttgcagacgtg	-
DOWN_murI(XbaI)	Reverse	ccctctagagggtccgtctcgagtccga	-

Supplementary Table 2 (cont.). Oligonucleotides and probes designed for the present work.

Unmarked deletion of <i>P. aeruginosa murI</i> gene			
EXTfw_murI	Forward	gtatcgccaagggtggagt	-
EXTrv_murI	Reverse	gaatggcttgatcgagtc	-
INTfw_murI	Forward	atccgaatcggtgctcta	-
INTrv_murI	Reverse	acaatacgcgcgtccagct	-
Unmarked deletion of <i>S. aureus murI</i> and <i>dat</i> genes			
UP_murI(MluI)	Forward	cccacgcgtggccgaaacaaaaacagta	-
UP_murI(NotI)	Reverse	cccgccggccgcggattcggtcatccttactt	-
DOWN_dat(NotI)	Forward	cccgccggccgcattttcatcatat	-
DOWN_dat(BglIII)	Reverse	cccagatctgcgaatctaaactcggt	-
EXTfw_murI	Forward	gcttgcctaaagggtattcc	-
EXTrv_murI	Reverse	gggccactcatacttatgac	-
INTfw_murI	Forward	tgtcgagggttgacagtag	-
INTrv_murI	Reverse	ctaactcacgagccgttc	-
EXTfw-seq-UP_murI	Forward	atgactgaacaatcagtcaa	-
EXTrv-seq-DOWN_murI	Reverse	tgtatggtgcctatgtaaagt	-
EXTfw_dat	Forward	gtcatgggtgacgtgacaac	-
EXTrv_dat	Reverse	gcaccacacctgctgaatcaag	-
INTfw_dat	Forward	tattcaagcaacgcgtgg	-
INTrv_dat	Reverse	agttgacgtgtaaattggcc	-
EXTfw-seq-UP_dat	Forward	gccgggtttaacagaagatg	-
EXTfw-seq-UP_dat	Forward	gccgggtttaacagaagatg	-
EXTrv-seq-DOWN_dat	Reverse	caattgcgggtctgcaatc	-

(*) These primers were used with D-Ala P-Taqman probe 6FAM-tccccgacacctgaagttagaaccagca-BBQ (6FAM, 6-carboxyfluorescein; BBQ, BlackBerry Quencher).

Supplementary References

1. Acosta, J. *et al.* Multidrug-resistant *Acinetobacter baumannii* harboring OXA-24 carbapenemase, Spain. *Emerg. Infect. Dis.* **17**, 1064–1067 (2011).
2. Russo, T. A. *et al.* The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. *Infect. Immun.* **78**, 3993–4000 (2010).
3. Lee, D. G. *et al.* Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. *Genome Biol.* **7**, R90 (2006).
4. Viedma, E., Juan, C., Otero, J. R., Oliver, A. & Chaves, F. Draft genome sequence of VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175, an epidemic high-risk clone. *Genome Announc.* **1**, e0011213 (2013).
5. Tomas, M. *et al.* Efflux pumps, OprD porin, AmpC beta-lactamase, and multiresistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Antimicrob. Agents Chemother.* **54**, 2219–2224 (2010).
6. Salunkhe, P. *et al.* A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. *J. Bacteriol.* **187**, 4908–4920 (2005).
7. Gómez-Zorrilla, S. *et al.* Impact of multidrug resistance on the pathogenicity of *Pseudomonas aeruginosa*: *in vitro* and *in vivo* studies. *Int. J. Antimicrob. Agents* **47**, 368–374 (2016).
8. Simon, R., Priefer, U. & Pühler, A. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. *Bio/Technology* **1**, 784–791 (1983).
9. Sambrook, J., Fritsch, E. F., & Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).
10. Monk, I. R., Shah, I. M., Xu, M., Tan, M. W. & Foster, T. J. Transforming the untransformable: application of direct transformation to manipulate genetically *Staphylococcus aureus* and *Staphylococcus epidermidis*. *mBio* **3**, e00277–11 (2012).

11. Vergara-Irigaray, M. *et al.* Relevant role of fibronectin-binding proteins in *Staphylococcus aureus* biofilm-associated foreign-body infections. *Infect. Immun.* **77**, 3978–3991 (2009).
12. Kreiswirth, B. N. *et al.* The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* **305**, 709–712 (1983).
13. Diep, B. A. *et al.* Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* **367**, 731–739 (2006).
14. Baba, T. *et al.* Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* **359**, 1819–27 (2002).
15. Baba, T. *et al.* Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. *J. Bacteriol.* **190**, 300–10 (2008).
16. Fitzgerald, J. R. *et al.* Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *J. Bacteriol.* **183**, 63–70 (2001).
17. Ben Zakour, N. L. *et al.* Genome-wide analysis of ruminant *Staphylococcus aureus* reveals diversification of the core genome. *J. Bacteriol.* **190**, 6302–6317 (2008).
18. Lowder, B. V. *et al.* Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. U S A* **106**, 19545–19550 (2009).
19. Hamad, M. A., Zajdowicz, S. L., Holmes, R. K. & Voskuil, M. I. An allelic exchange system for compliant genetic manipulation of the select agents *Burkholderia pseudomallei* and *Burkholderia mallei*. *Gene* **430**, 123–131 (2009).
20. Hoang, T. T., Karkhoff-Schweizer, R. R., Kutchma, A. J. & Schweizer, H. P. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* **212**, 77–86 (1998).

21. Arnaud, M., Chastanet, A. & Debarbouille, M. New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl. Environ. Microbiol.* **70**, 6887–6891 (2004).